IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Serial No.: continuation of 09/298,084 Express Mail Label: EL 717 750 047 US

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For: *CONTROLLED RELEASE OF NON-HEPARIN BINDING GROWTH FACTORS FROM HEPARIN CONTAINING MATRICES*

BOX PATENT APPLICATION
Assistant Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

Prior to consideration of the claims in this application, please enter the following amendment to the claims and specification.

Amendment

In the Specification

Page 1, first paragraph, insert --This is a continuation of U.S. Serial No. 09/298,084 filed April 22, 1999, now abandoned.--

Please replace the paragraph on page 6, lines 13-19, with the following paragraph.

The peptides of the invention that bind heparin with high affinity have a characteristic amino acid domain that will not elute from a heparin-affinity column at less than 140 mM NaCl. While many potential peptides exist, the inventors have identified several peptide sequences in particular. These are exemplified in the amino acid sequences identified in SEQ ID NO: 1, SEQ

ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, and SEQ ID NO: 5. Many other peptides may be used apart from the specifically enumerated sequences here.

In the Claims

1. (Amended) A [matrix] drug delivery composition comprising:

a substrate [capable of providing attachment of a heparin-binding peptide];

a peptide comprising a [binding] domain that binds heparin or heparin-like compounds with high affinity affinity,

wherein the peptide is covalently bound to the substrate so that the heparin binding domain is able to bind to heparin or heparin-like compounds;

heparin or a heparin-like polymer; and

a protein growth factor or a peptide fragment thereof having a domain that binds heparin with low affinity, wherein low affinity is defined as not binding with heparin at a NaCl concentration of between about 25 mM and 140 mM.

Please cancel claim 2.

3. (Three times Amended) The [matrix] composition of claim 1 wherein the domain of the growth factor or peptide fragment thereof is further defined as comprising a length of about 8 to 30 amino acid residues comprising at least 2 basic amino acid residues, a ratio of basic to acidic amino acid residues of at least 2, and a ratio of hydrophobic amino acid residues to basic amino acid residues of at least 0.67.

4. (Amended) The [matrix] composition of claim 3 wherein the basic amino acid residues are K or R.

5. (Amended) The [matrix] composition of claim 3 wherein the acidic amino acid residues are further defined as D or E.

6. (Amended) The [matrix] composition of claim 3 wherein the hydrophobic amino acid residues are further defined as A, V, F, P, M, I, or L or C when C is involved in a disulfide bond.

7. (Three times Amended) The [matrix] composition of claim 1 wherein the growth factor or peptide fragment thereof is selected from the group consisting of neurturin, persephin, IGF-1A, IGF-1β, EGF, NGFβ, NT-3, BDNF, NT-4, [TGF-β2,] TGF-β3, [or] and TGF-β4.

Please cancel claims 8-19.

20. (Amended) The [matrix] composition of claim 66 wherein the substrate comprises fibrin.

21. (Amended) The [matrix] composition of claim 66 wherein the substrate comprises a synthetic polymer hydrogel.

24. (Amended) The [matrix] composition of claim 64 wherein the heparin or heparin-like polymer has a molecular weight between about 3,000 and 10,000,000 Daltons.

25. (Amended) The [matrix] composition of claim 64 wherein the heparin-like polymer is a polysaccharide having a molecular weight between about 3,000 and 10,000,000 Daltons, and having at least one negative charge per two saccharide rings and no more than one positive charge per ten saccharide rings.

26. (Amended) The [matrix] composition of claim 64 wherein the heparin-like polymer is selected from the group consisting of dextran sulfate, chondroitin sulfate, heparin sulfate, fucan, alginate, [or] and a derivative thereof.

27. (Three times Amended) The [matrix] composition of claim 1 wherein the molar ratio of heparin or heparin-like polymer to growth factor or peptide fragment thereof is at least one.

Please cancel claims 29-56.

57. (Amended) [A] The composition of claim 1 in a vascular graft [comprising a matrix capable of supporting cell adhesion, said matrix comprising

bound heparin or heparin-like polymer and a growth factor having a low binding affinity for heparin].

58. (Amended) [An] The composition of claim 1 in an article for treatment of dermal wounds [comprising a matrix capable of supporting cell adhesion, said matrix comprising bound heparin or heparin-like polymer and a growth factor having low binding affinity for heparin].

59. (amended) The [article] composition of claim 58, wherein the growth factor is TGF-β.

Please cancel claim 60.

61. (Amended) [An] The composition of claim 1 in an implantable sterilized composition [comprising

a matrix capable of supporting cell adhesion, said matrix comprising bound heparin or a heparin-lie polymer and a growth factor or peptide fragment thereof having low binding affinity for heparin].

62. (Amended) A method for providing controlled release of a growth factor comprising:

preparing a [matrix comprising a growth factor having a domain with a low affinity for binding heparin and bound heparin or heparin-like polymer] composition comprising

a substrate,

a peptide comprising a domain that binds heparin or heparin-like compounds, wherein the peptide is covalently bound to the substrate so that the heparin binding domain is able to bind to heparin or heparin-like compounds,

heparin or a heparin-like polymer, and

a growth factor or a peptide fragment thereof having a domain with low affinity for binding heparin and bound heparin or heparin-like polymer, wherein low affinity is defined as not binding with heparin at a NaCl concentration of between about 25 mM and 140 mM; and

placing the composition on a wound in need thereof.

63. (Amended) The method of claim 62, wherein the growth factor or a peptide fragment thereof is released by dissociation of [a component of the matrix] the growth factor from the heparin or heparin-like polymer.

Please add new claims 64 and 65.

64. The composition of Claim 1, wherein the heparin or heparin-like compound is non-covalently attached to the peptide.

65. The composition of Claim 1 wherein the substrate is selected from the group comprising fibrin, collagen and synthetic polymer hydrogels.

Remarks

The following comments are in response to rejections made in the parent application in view of the prior art.

Rejection Under 35 U.S.C. § 103

Claims 1, 3-6, 20, 24-27, and 60-66 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Schroeder-Tefft et al., *J. Controlled Release* 48:29-33 (1997) (Schroeder-Tefft) in view of Kwon et al., *J. Controlled Release* 22: 83-94 (1992) (Kwon), Cardin & Weintraub, *Arteriosclerosis* 9:1 (21-32) (1989) (Cardin), Darling & Fahnestock, *Biochemistry* 27:6686-6692 (1988) (Darling), DeBlois et al., *Biomaterials* 15:9 (665-672) (1992) (DeBlois), and Powell et al., *Brain Research* 515: 309-311 (1990) (Powell).

Claims 21 and 26 were rejected under 35 U.S.C. § 103(a) as being obvious over Schroeder-Tefft in view of Kwon, Cardin, Darling, DeBlois, and Powell and in further view of Alberts et al., *Molecular Biology of the Cell* (1994) (Alberts).

Claims 57 and 58 were rejected under 35 U.S.C. § 103(a) as being obvious over Schroeder-Tefft in view of Kwon, Cardin, Darling, DeBlois, and Powell and in further view of Levi-Montalcini et al., *TINS* 19:11 (514-520) (1996) (Levi-Montalcini).

The present invention

The present invention is related to the controlled delivery of growth factors, which bind to heparin or heparin-like compounds with **low affinity – i.e., they are "non-heparin binding-growth factors**. The claims are directed to compositions, specific uses for the compositions and methods for delivering these growth factors. The controlled delivery compositions contain a substrate, a peptide with a domain the binds with heparin or heparin-like compounds with high affinity, heparin or a heparin-like compound, and a growth factor that binds with heparin with low affinity. The high-affinity peptide is **covalently** attached to the substrate. In turn, heparin or heparin-like compounds bind to the peptide and are immobilized on the substrate, either by covalent bonds or non-covalent bonds (see e.g. Example 3, at page 17, lines 14-18). Non-heparin binding peptides are then loosely associated with the heparin and released upon use.

Schroeder-Tefft

Schroeder-Tefft does not teach or suggest grafting heparin to a collagen substrate to create a composition for the controlled delivery of growth factors. In fact, Schroeder-Tefft *teaches away* from grafting heparin to a substrate. Schroeder-Tefft teaches that TGF-β2 should be complexed to heparin, and then the heparin/TGF-β2 complex should be **mixed** with collagen (see page 295, col. 1). Since collagen also binds with heparin, this order, i.e. first binding the heparin to TGF-β2 (in the absence of collagen), **prevents** the binding of heparin with collagen and maximizes the binding of heparin with TGF-β2. Further, nowhere does Schroeder-Tefft teach or suggest that the drug delivery composition should include a peptide which links to heparin (or a heparin-like compound), as required by the claims. Schroeder-Tefft specifically

uses a tight binding of heparin to the substance to be released, to stabilize the protein; not to release it.

Kwon

Kwon is directed at studying the viability of using ion exchange as a release mechanism for macromolecular delivery from microspheres (page 84, col. 1). Kwon neither teaches nor suggests covalently binding a peptide to a substrate with heparin binding sites. Further, Kwon neither teaches nor suggests including a peptide with heparin binding domains to help deliver a growth factor with a domain that binds with heparin with low affinity.

Cardin

Cardin identifies heparin binding regions in proteins.

Darling

Darling is directed at determining the biological role of the different subunits of NGF. Nowhere does Darling teach or suggest the claimed compositions, uses or methods for delivery of growth factors that bind heparin with low affinity.

DeBlois

DeBlois is directed at the delivery of FGF, a heparin-binding growth factor (see page 665, col. 2). It does not teach or suggest compositions or methods for the delivery of growth factors that bind to heparin with low affinity.

Powell

Powell controlled release of NGF from ethylene-vinyl acetate copolymer (EVAc) implants that contain NGF. Powell does not teach or suggest that release of a growth factor with

a domain that bind to heparin with low affinity can be controlled through the use of a substrate and a peptide, which contains heparin binding domains.

Alberts

Alberts is directed to a general discussion of glycosaminoglycans.

Levi-Montalcini

Levi-Montalcini provides a general disclosure of NGF's role in the nervous, immune and endocrine systems.

The combined references

Even if these references were combined, they would not teach or suggest the claims compositions, uses for the compositions, or methods to one of ordinary skill in the art. None of these references teaches binding heparin to a substrate. In fact, Schroeder-Tefft teaches away from binding heparin with the substrate, by emphasizing the importance of the order of first binding heparin to TGF-β2 and subsequently mixing the heparin/ TGF-β2 complex with the substrate. Nor do these references teach or suggest that the using a substrate, a peptide with a domain the binds with heparin or heparin-like compounds, heparin or a heparin-like compound, and a growth factor that binds with heparin with low affinity will provide for the controlled release of the growth factor.

Allowance of claims 1, 3-16, 18-27, 57-59, and 61-65, as amended, is respectfully solicited.

Respectfully submitted,

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Date: May 3, 2001

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Certificate of Mailing Under 37 C.F.R. § 1.8(a)

I hereby certify that this paper, along with any paper referred to as being attached or enclosed, is being deposited with the United States Postal Service on the date shown below with sufficient postage as first-class mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Patrea L. Pabst

Date: May 3, 2001

Clean Version of Amended Claims
Pursuant to 37 C.F.R. § 1.121(c)(1)(ii)

1. (Amended) A drug delivery composition comprising:

a substrate;

a peptide comprising a domain that binds heparin or heparin-like compounds with high affinity affinity,

wherein the peptide is covalently bound to the substrate so that the heparin binding domain is able to bind to heparin or heparin-like compounds;

heparin or a heparin-like polymer; and

a protein growth factor or a peptide fragment thereof having a domain that binds heparin with low affinity, wherein low affinity is defined as not binding with heparin at a NaCl concentration of between about 25 mM and 140 mM.

Please cancel claim 2.

3. (Three times Amended) The composition of claim 1 wherein the domain of the growth factor or peptide fragment thereof is further defined as comprising a length of about 8 to 30 amino acid residues comprising at least 2 basic amino acid residues, a ratio of basic to acidic amino acid residues of at least 2, and a ratio of hydrophobic amino acid residues to basic amino acid residues of at least 0.67.

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58. (Amended) The composition of claim 1 in an article for treatment of dermal wounds.

59. (amended) The composition of claim 58, wherein the growth factor is TGF-β.

61. (Amended) The composition of claim 1 in an implantable sterilized composition.

62. (Amended) A method for providing controlled release of a growth factor comprising:

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64. The composition of Claim 1, wherein the heparin or heparin-like compound is non-covalently attached to the peptide.

65. The composition of Claim 1 wherein the substrate is selected from the group comprising fibrin, collagen and synthetic polymer hydrogels.

Marked Up Version of Amended Specification Paragraphs

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Page 1, first paragraph, insert –This is a continuation of U.S. Serial No. 09/298,084 filed April 22, 1999, now abandoned.--

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